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Research report

# Diverse effect of different odor stimuli on behavior and Fos protein production in the olfactory system neurogenic region of adult rats

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## HIGHLIGHTS

- Single exposure to artificial or cat odor induces a defensive response in rats.
- In rats exposed to the cat odor Fos expression is elevated.
- In rats exposed to the artificial odor Fos expression is elevated.
- In cat odor stimulated rats are greater changes in anxiety level.
- SVZ/RMS cells have the prerequisites necessary for Fos signal transduction cascade.

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## ABSTRACT

Previously it has been demonstrated that processes of postnatal neurogenesis in the olfactory system neurogenic region—the subventricular zone (SVZ), rostral migratory stream (RMS), and olfactory bulb (OB) can be significantly altered by different factors of an environment. However, the mechanisms involved in regulation of neurogenesis by exogenous factors in the olfactory system remain unclear. The purpose of the present study was to contribute to the understanding of these mechanisms by immunohistochemical assessment of Fos protein induction in areas of adult neurogenesis. To evaluate the coordinate activation of Fos production in neurons of the olfactory system neurogenic region, a brief exposure to artificial odor (eau de Cologne) or naturalistic odor (cat odor) has been used in alert rats. Our results revealed that the effects of these odors are easily distinguishable at both the behavioral and the morphological level. Cat odor induced greater changes in anxiety level, and produced typical pattern of Fos activation in the accessory olfactory bulb (AOB), a brain region associated with defensive behavior. An important finding is, that next to distinct Fos expression in the OB and the AOB, Fos positive cells have been found also within the SVZ/RMS of the odor stimulated rats. Interestingly, Fos expression in the RMS was detected only after exposure to artificial odor stimulus. These results provide new evidence that some SVZ/RMS cells have complete prerequisites necessary for the Fos signal transduction cascade.



## 1. Introduction

The mammalian forebrain contains two neurogenic regions that continuously produce new neurons in adulthood; the subgranular zone (SGZ) of the hippocampal dentate gyrus, and the subventricular zone (SVZ) along the walls of the lateral ventricles [1] and [2]. Both of these areas retain astrocyte-like neural stem cells that appear to be derived from the embryonic radial glial lineage [3]. Neuroblasts generated in the SGZ differentiate into dentate granule cells. Those born in the adult SVZ undergo chain migration within longitudinal channels formed by specialized astrocytes in the rostral migratory stream (RMS) to reach the olfactory bulb (OB) [1]. There they differentiate largely into granule or for a small part into periglomerular cells, both of which function as local interneurons [4] and [5]. Olfactory bulb granule cells, which lack axons and modulate the activity of mitral and tufted cells through dendrodendritic connections, are gradually eliminated from the adult OB through programmed cell death and are continually replaced by new neurons [6].

Although neurogenesis in the olfactory system neurogenic region, i.e., in the SVZ/RMS/OB system occurs continuously throughout life, its individual processes may be influenced by

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various exogenous factors. For example, Orendáčová et al. (2009) have shown significant age- and dose-dependent changes in proliferating precursor cell number within the rat RMS after electromagnetic radiation [7]. In another study, analyzing the effect of ionizing radiation dramatic reduction in cell proliferation accompanied by reactive gliosis has been demonstrated within the SVZ/RMS/OB [8]. Recent study from our laboratory has shown that stressful experience (maternal deprivation) imposed on P1–P7 rats induces a decrease in proliferating cell numbers and causes premature occurrence and differentiation of cells producing a signaling molecule of nitric oxide (NO) in the RMS [9]. However, mechanisms involved in regulation of neurogenesis by exogenous factors remain largely unknown.

Over the past decade many studies have used c-Fos immunohistochemistry to explore the patterns of brain activation in laboratory animals exposed to various exogenous factors with special emphasis on stressful or anxiogenic stimuli [10]. Results have implicated certain key brain regions in the emotional states of anxiety and fear and the control of defensive behavior that comes into play during these states. However, the pattern of activation seems to be dependent on the anxiety model employed.

Several strategies have evolved in eukaryotic cells for regulating gene expression in response to extracellular cues. The first set of genes activated by external signals is that which does not require de novo synthesis of proteins (immediate early genes or IEGs). The induction of IEGs is rapid and transient. The most well-studied of these genes is probably *c-fos.*

The immediate early gene *c-fos* is rapidly and transiently expressed in neurons in response to stimulation [11] and [12]. Transcriptional activation of the gene occurs within minutes of stimulation, with the accumulation of mRNA reaching its peak approximately 30 to 40 min later. The gene encodes for the nuclear protein Fos. Like its mRNAs, this protein is relatively short-lived (Fos has a half-life of approximately 2 h) and thus has the characteristics of a signaling system. Fos forms heterodimeric complex with other nuclear proteins of the Jun family (encoded by the family of *jun* genes). Both *fos* and *jun* are members of gene families in which the zipper motif is conserved. Many different types of stimuli have been shown to elicit *c-fos* expression in the nervous system. This has led to the notion that neuronal activity and the presence of Fos may be correlated. Thus, *c-fos* is involved in the signal transduction cascade that links extracellular events to long-term intracellular adaptations. Expression of the gene is typically measured by either Northern blot analysis or in situ hybridization, while the protein is usually visualized using immunohistochemical techniques [13], [14], and [15].

Recently there has been increasing interest in ethological tests of stress and anxiety which involve exposing laboratory animals to anxiogenic stimuli that closely mimic those seen in the wild [16], [17], and [18]. It can be argued that the use of such naturalistic stimuli allows for greater precision in the evocation of emotional states and defensive behaviors, thereby leading to improved modeling and analysis of fear and anxiety states [19] and [20].

In the present study we intended to examine if single exposure of adult rats to various odor stimuli could induce immediate changes in Fos positivity within the olfactory system neurogenic region, the SVZ/RMS/OB system. Expression of the Fos protein was used to evaluate the coordinate activation of olfactory system neurogenic region neurons by brief exposure to artificial odor (eau de Cologne) and naturalistic odor (cat odor) in alert rats.

Adult rats exposed to the stressful odor stimuli show species typical defense behavior, including freezing, flight, avoidance, and risk assessment [21]. Partial predator stimuli, such as predator odor, may represent a potential threat and these stimuli also elicit some of the same defense behaviors, as does actual predator presence [22], [23], and [24]. Therefore the further aim of our study was to assess anxiety in adult rats using a simple test battery that included open field test and emergence test.

Nitric oxide, a diffusible intracellular and intercellular messenger has been confirmed to play an important role in control of adult neurogenesis [25]. Very recently, we have brought immunohistochemical evidence showing that nitrergic cells of the RMS display characteristics of mature neurons and form synaptic connections [26]. These results suggest that NO producing cells in the RMS could be involved in a neuronal circuitry with potential impact on regulation of neurogenesis. Coupling of the nitric oxide synthase activity known to produce NO seems to be operative in the mechanism responsible for Fos expression, thus suggesting that NO may be part of the N-methyl-D-aspartate (NMDA) receptor-coupled mechanism responsible for activation of *c-fos* expression [27]. To reveal possible colocalization of Fos and NO within the olfactory system neurogenic region we have investigated the morphology and distribution of NADPH-diaphorase (NADPH-d) positive and Fos positive neurons.

## 2. Material and Methods

### 2.1. Subjects

Twenty-four (3–4 months) male Wistar rats were used in this study. The mean weight of the rats was 332 g (range 323–360 g). Rats were housed individually in small plastic tubs in a temperature-controlled colony room (21 ± 2◦C) on a reverse light/dark cycle (lights on from 7:00 PM to 7:00 AM). Food and water were available *ad libitum*. Behavioral testing was performed during the dark cycle. All rats were handled extensively for 14 days prior to the start of experimentation. Rats were tested sequentially on the behavioral tasks with a minimum of 1 week between each test.

Experimental protocols were approved by the Institutional Ethical Committee, in accordance with current Slovak Republic legislation.

### 2.2. Open field (OF)

The OF was a plastic gray rectangular box opened at the top (60 × 45 × 35 cm). A piece of fabric collar impregnated with cat fur odor, artificial odor, or clean without odor (3 × 3 cm) was placed and fixed on the floor in the bottom right corner. A video camera was suspended from the ceiling above the arena (1.20 m above the surface of the OF) and connected to a computer. Rats were divided into 3 groups: (1) control rats: n = 8, fabric collar without odor; (2) artificial odor rats: n = 8, fabric collar scented with eau de Cologne; and (3) cat odor rats: n = 8, fabric collar worn by cat. Initially, the rat was placed in the middle of OF and tested for 8 min. During testing, the experimenter remained outside the testing room. Automatic analysis of (i) the distance traveled and (ii) the mean velocity to measure the locomotor activity was accomplished using Ethovision XT 7. Subsequent manual video analysis by an experimenter blind to group assignment scored: (iii) number of contacts—the number of rats nose or mouth touching the collar; (iv) grooming—preening or licking in a typical fashion; (v) rearing—standing on hind legs with both forelegs off the ground; (vi) freezing; (vii) the number of crossing the middle point of OF; (viii) the time spent by the walls of OF, and (ix) defecation to measure the anxiety level. At the completion of each trial the OF was cleaned with a tap water solution containing 50% ethanol. To prevent cross-contamination on the experimental day, control rats were run first. The arena was also carefully cleaned at the completion of each groups and the testing room well-ventilated.